

Fig. 1

1B

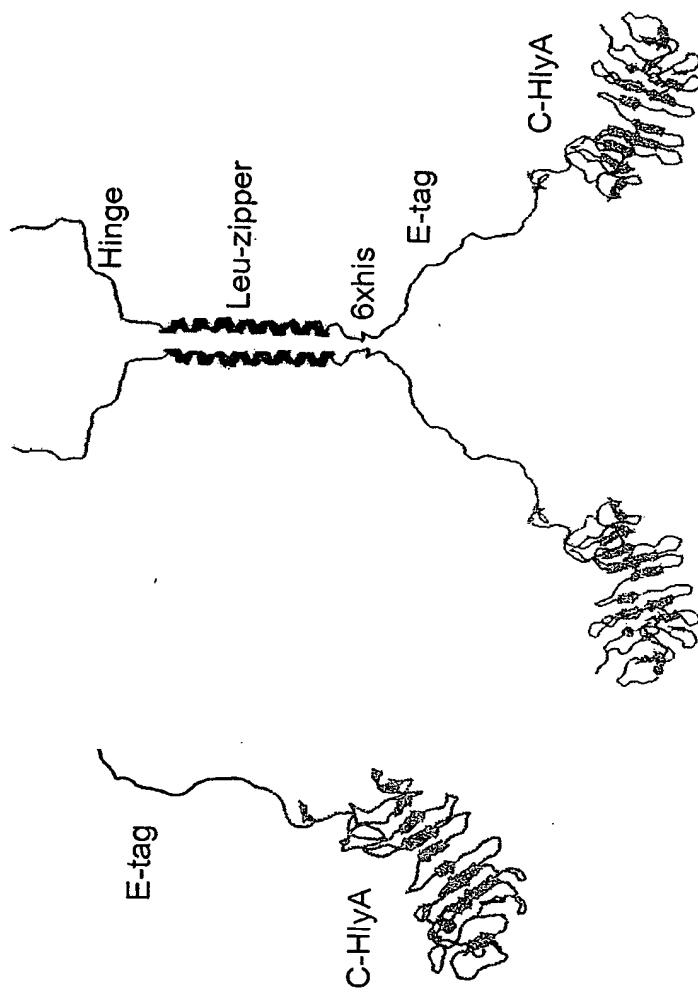


Fig. 1 (cont.)

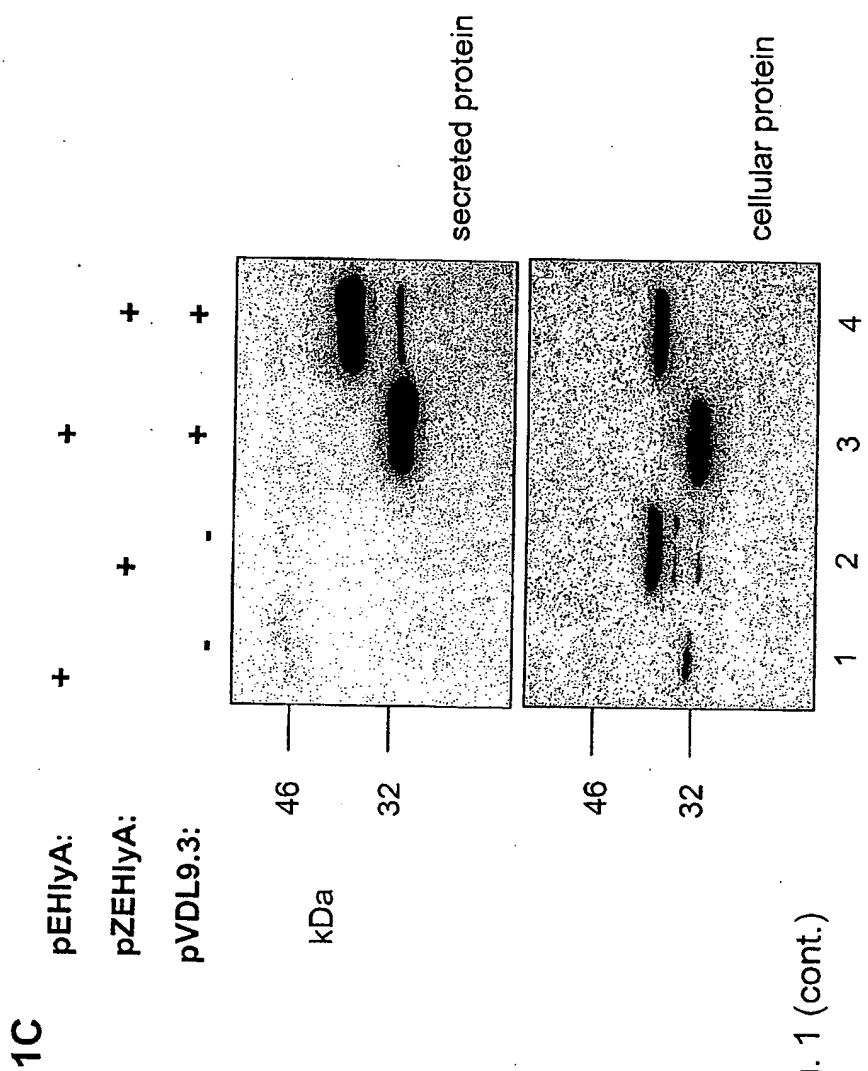


Fig. 1 (cont.)

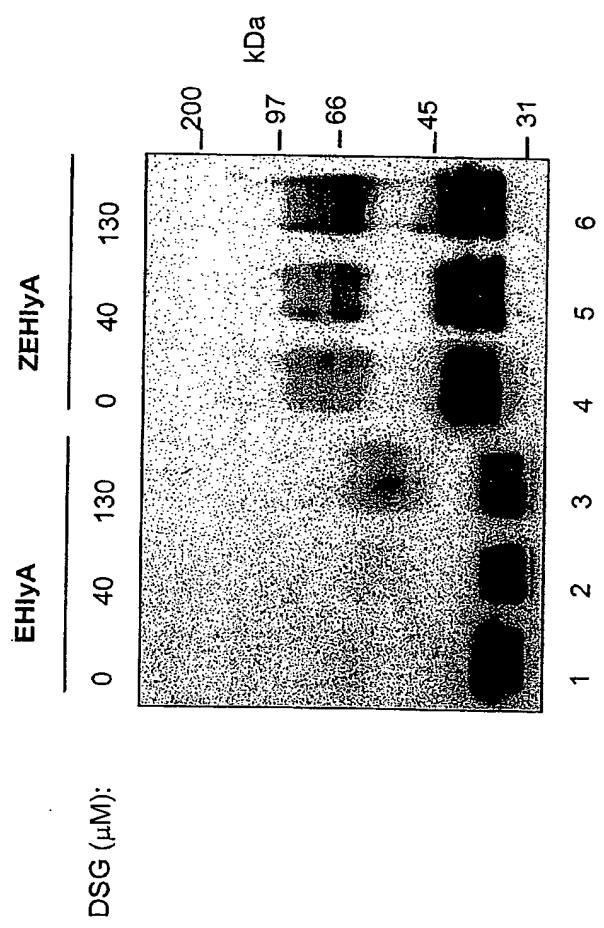


Fig. 2

3A

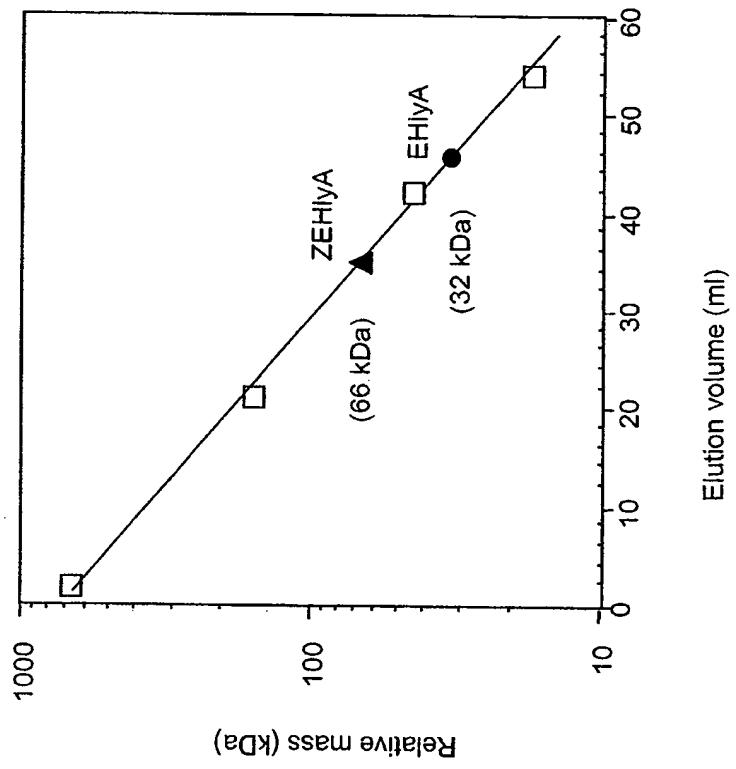
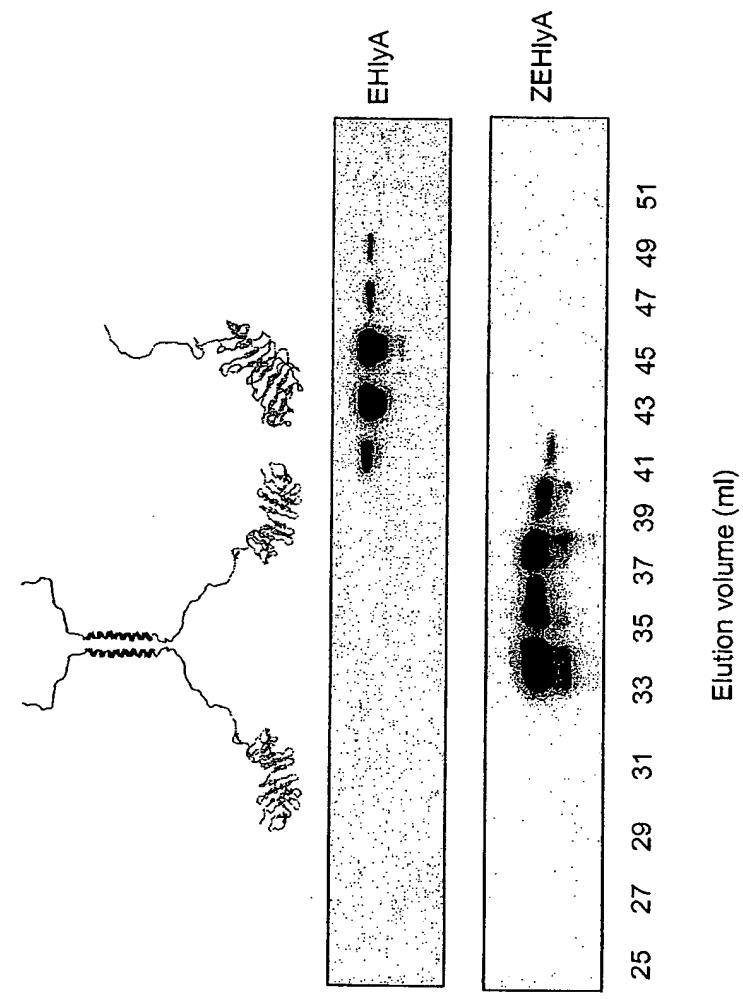


Fig. 3

3B

Fig. 3 (cont.)



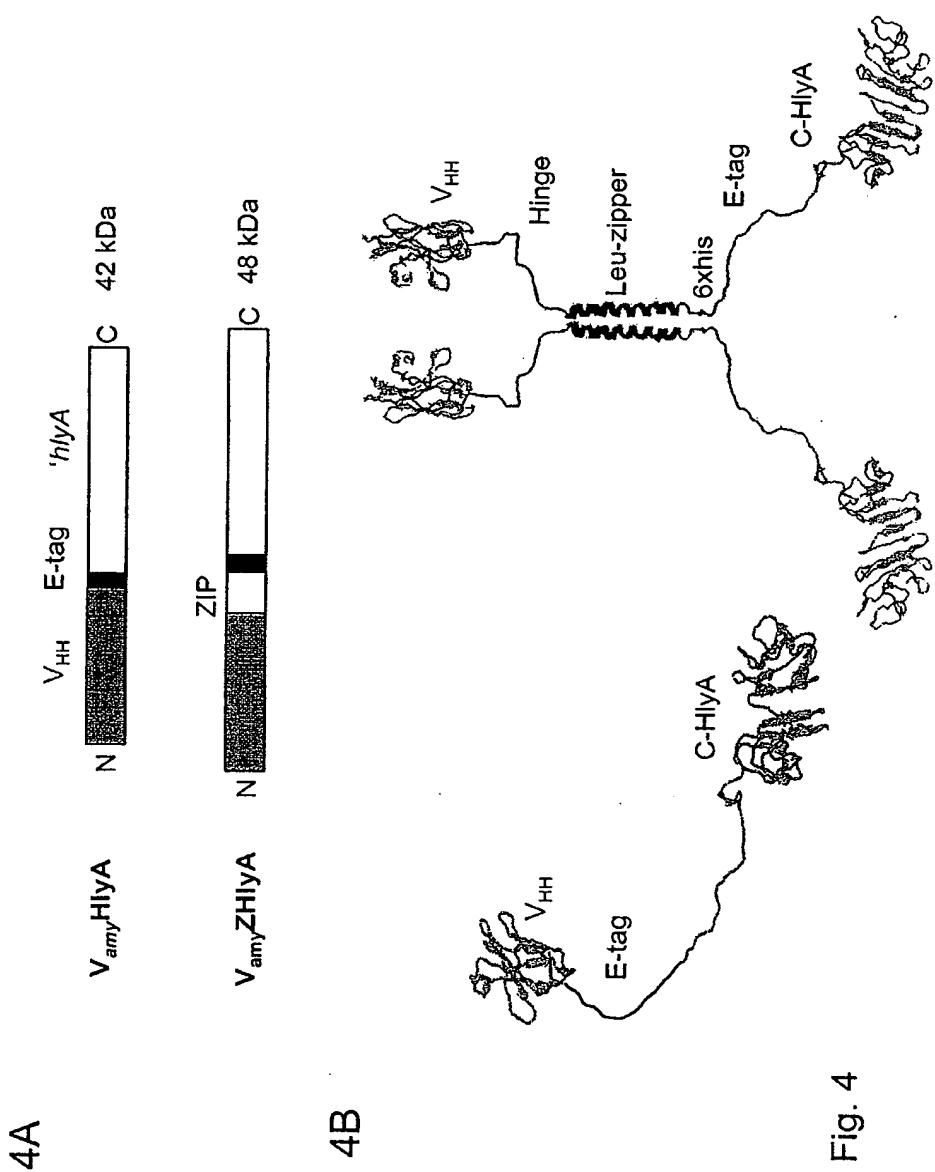


Fig. 4

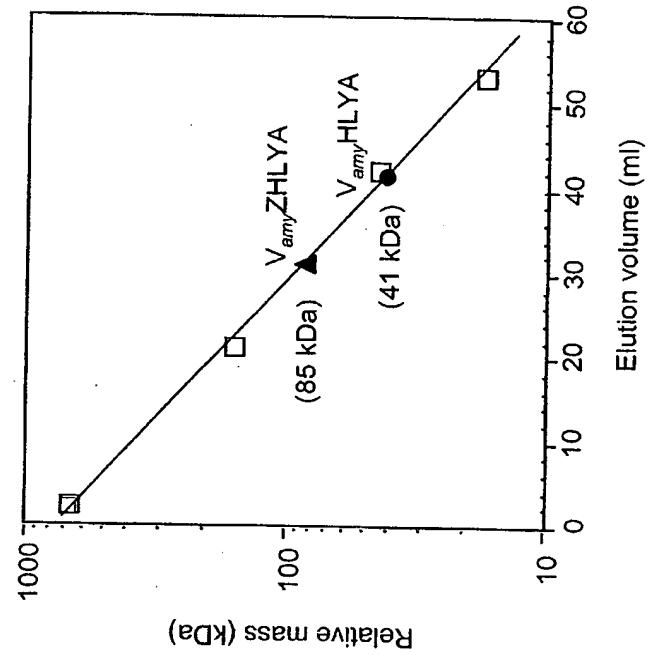
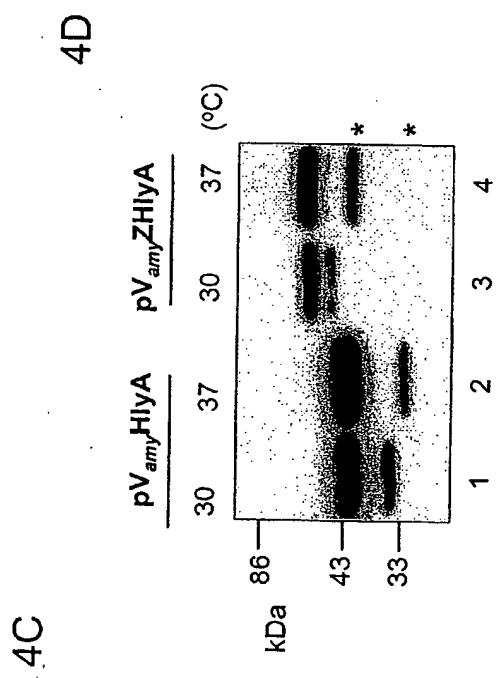
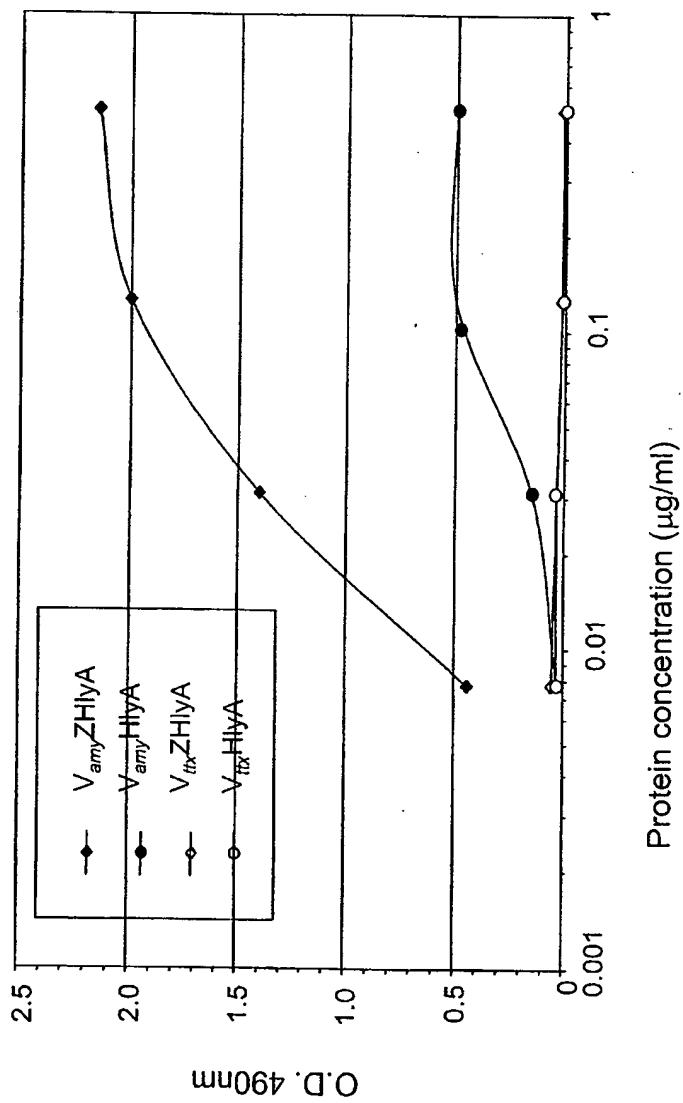


Fig. 4 (cont.)

Fig. 5



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pZEH1yA map

With 16 restriction enzymes: ECORI PSTI SALI HINDIII BAMHI NOTI SACI
SACII SPHI KPNI XBAI NHEI NDEI

AGCGGATAACAATTCACACAGGAAACAGCT (sequence before the ATG)

Fig. 6

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GGTAATGACAAGTTGTACGGCAGTGAGGGAGCAGACCTGTTGATGGCGGAGAAGGAAAT
301 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 360
CCATTACTGTTAACATGCCGTCACTCCCTCGTCTGGACGAACTACCGCCTCTTCCCTTA

a G N D K L Y G S E G A D L L D G G E G N -

GATCTTCTGAAAGGTGGATATGGTAATGATATTTATCGTTATCTTCAGGATATGGCAT
361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 420
CTAGAAGACTTTCCACCTATAACCATTACTATAAAAGCAATAGAAAGTCCTATACCGGTA

a D L L K G G Y G N D I Y R Y L S G Y G H -

CATATTATTGACGATGAAGGGGGAAAGACGATAAACTCAGTTAGCTGATATAGATTTC
421 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
GTATAATAACTGCTACTTCCCCCTTCTGCTATTTGAGTCAAATCGACTATATCTAAAG

a H I I D D E G G K D D K L S L A D I D F -

CGGGACGTTGCCTTAAGCGAGAAGGGAAATGACCTCATTATGTATAAAGCTGAAGGTAAT
481 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 540
GCCCTGCAACGGAAATTCGCTTCCCTACTGGAGTAATAACATATTCGACTTCCATTA

a R D V A F K R E G N D L I M Y K A E G N -

GTTCTTCTATTGCCACAAAATGGTATTACATTAAAAACTGGTTGAAAAAGACTCA
541 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 600
CAAGAAAAGATAACCGGTGTTTACCATATGTAAATTGACCAAACCTTTCTCAGT

a V L S I G H K N G I T F K N W F E K E S -

GATGATCTCTAATCATCAGATAGAGCAGATTGGATAAAAGACGGCAGGGTAATCACA
601 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 660
CTACTAGAGAGATTAGTAGTCTATCTCGTCTAAAACATTCTGCCGTCCCATTAGTGT

a D D L S N H Q I E Q I F D K D G R V I T -

CCAGATTCTCTAAAAAGCATTGAAATATCAGCAGAGTAATAACAAGGTAAGTTATGTG
661 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 720
GGTCTAAGAGAATTTCGTAACCTATAGTCGTCATTATTGTTCCATTCAACAC

a P D S L K K A F E Y Q Q S N N K V S Y V -

TATGGACATGATGCATCAACTTATGGGAGCCAGGACAATCTTAATCCATTAAATTAGAA
721 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 780
ATACCTGTACTACGTAGTTGAATACCCCTCGGTCTGTTAGAATTAGGTAATTACTT

a Y G H D A S T Y G S Q D N L N P L I N E -

Fig. 6 (cont.)

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PstI

ATCAGCAAAATCATTCAGCTGCAGGTAACCTCGATGTTAAGGGAGGAAGATCTGCCGCT
781 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 840
TAGTCGTTTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTCTAGACGGCGA

a I S K I I S A A G N F D V K E E R S A A -

NdeI

TCTTTATTGCAGTTGTCCGGTAATGCCAGTGATTTTCATATGGACGGAACTCAATAACT
841 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 900
AGAAAATAACGTCAACAGGCCATTACGGTCACTAAAAAGTATACTGCCTTGAGTTATTGA

a S L L Q L S G N A S D F S Y G R N S I T -

TTGACAGCATCAGCATAA
901 -----+----- 918
AACTGTCGTAGTCGTATT

a L T A S A * -

Cutting enzymes:

AccI BamHI EcoRI HindIII NdeI PstI SacI SalI
SmaI XbaI

Not cutting enzymes:

KpnI NcoI NheI NotI SacII SphI

Fig. 6 (cont.)

13/30

pZEHLYA2SD map

With 11 restriction enzymes: ECORI BGLII BAMHI NCOI NHEI SFI I SALI XMAI XBAI EAGI SALI HINDIII

BglII EcoRI

1 ATGAATACGAATTAGATCTGAATTGGGCCCTCGAAAAATTAAATACGACTCACTATAGG
1 TACTTATGCTTAAATCTAGACTTAAGCCGGGAAGCTTTAATTATGCTGAGTGATATCC 60

XbaI NcoI

61 GAGACCACAAACGGTTCCCTAGAAAATAATTTGTTAACCTTAAGAAGGAGATATATC
61 CTCTGGTGTGCAAAGGGAGATCTTATTAAAACAATTGAAATTCTCCTCTATATAG 120

NheI SfiI SalI

121 CATGGCTAGCACGGCCTCGGGGGCGCGTCGACGTCCGGTCCGAAGCCTTCACTCC
121 GTACCGATCGTGCAGGAGCCCCGGCGCAGCTGCAGGCCAGGCTTCGGAAGGTGAGG 180

b M A S T A S G A A S T S G G P K P S T P -
|=> Ig hinge

XmaI

181 GCCCGGGCTTCCCGTATGAAACAGCTGGAAGACAAAGTAGAGGAGCTCCTAGCAAGAA
181 CGGGCCCAGAAGGGCATACTTGTCGACCTCTGTTCATCTCCTCGAGGAATCGTTCTT 240

b P G S S R M K Q L E D K V E E L L S K N -
|=> Leu-zipper GCN4

XbaI HindIII

241 CTACCATCTAGAAAACGAGGTAGCTCGTCTGAAAAAGCTTGTGGTGAACGTGGTGGTCA
241 GATGGTAGATCTTGCTCCATCGAGCAGACTTTCGAACAAACCACTTGCACCAACAGT 300

b Y H L E N E V A R L K K L V G E R G G H -
|=>

SalI XmaI BamHI

301 CCATCACCATCACCATGCGTCGACGCCGGGGTGCGCCGGTGCCTATCCGGATCCGCT
301 GGTAGTGGTAGTGGTACCCAGCTGCAGGCCACGGCATAGGCCTAGGCAG 360

Fig. 7

b H H H H H A S T P G G A P V P Y P D P L -
 6xhis tag |=> E-tag
 EagI
 |
 361 GGAACCGGCCGGGAAAATTCTCTTGCATAAAATGTATTATCCGGTGGAAAAGGTAATGA
 -----+-----+-----+-----+-----+-----+ 420
 CCTTGGCCGGCCCCTTTAAGAGAACGATTACATAATAGGCCACCTTTCCATTACT

b E P A G E N S L A K N V L S G G K G N D -
 |=> C-hlyA
 421 CAAAGTTGTACGGCAGTGAGGGAGCAGACCTGCTTGTGATGGCGGAGAAGGGAATGATCTTCT
 -----+-----+-----+-----+-----+-----+ 480
 GTTCAACATGCCGTCACTCCCTCGTCTGGACGAACCTACCGCTCTCCCTACTAGAAGA

b K L Y G S E G A D L L D G G E G N D L L -
 481 GAAAGGTGGATATGGTAATGATATTATCGTTATCTTCAGGATATGCCATCATATTAT
 -----+-----+-----+-----+-----+-----+ 540
 CTTTCACCTATACCACTACTATAAATAGCAATAGAAAGTCCTATACCGTAGTATAATA

b K G G Y G N D I Y R Y L S G Y G H H I I -
 541 TGACGATGAAGGGGGAAAGACGATAAAACTCAGTTAGCTGATATAGATTCCGGGACGT
 -----+-----+-----+-----+-----+-----+ 600
 ACTGCTACTTCCCCCTTCTGCTATTGAGTCAAATCGACTATATCTAAAGGCCCTGCA

b D D E G G K D D K L S L A D I D F R D V -
 601 TGCCTTAAGCGAGAAGGGATGACCTCATATTGTATAAAGCTGAAGGTAATGTTCTTC
 -----+-----+-----+-----+-----+-----+ 660
 ACGGAAATTGCTCTCCCTACTGGAGTAATACATATTCGACTTCATTACAAGAAAG

b A F K R E G N D L I M Y K A E G N V L S -
 661 TATTGGCCACAAAATGGTATTACATTAAAACTGGTTGAAAAGAGTCAGATGATCT
 -----+-----+-----+-----+-----+-----+ 720
 ATAACCGGTGTTTACCATATGTAAATTGACCAAACCTTCTCAGTCTACTAGA

b I G H K N G I T F K N W F E K E S D D L -
 721 CTCTAACATCAGATAGAGCAGATTTGATAAAAGACGGCAGGGTAATCACACCAGATT
 -----+-----+-----+-----+-----+-----+ 780
 GAGATTAGTAGTCTATCTCGTCTAAAACATTCTGCCGTCCCATTAGTGTGGTCTAAG

b S N H Q I E Q I F D K D G R V I T P D S -
 781 TCTTAAAAAAGCATTTGAATATCAGCAGAGTAATAACAAGGTAAGTTATGTGTATGGACA
 -----+-----+-----+-----+-----+-----+ 840
 AGAATTTCGTAACCTATAGTCGTCATTATTGTTCCATTCAATACACATACTGT

Fig. 7 (cont.)

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b L K K A F E Y Q Q S N N K V S Y V Y G H -
TGATGCATCAACTTATGGGAGGCCAGGACAATCTTAATCCATTAAATGAAATCAGCAA
841 -----+-----+-----+-----+-----+-----+-----+ 900
ACTACGTAGTTGAATACCCTCGGTCTGTTAGAATTAGGTAATTAATTACTTAGTCGTT

b D A S T Y G S Q D N L N P L I N E I S K -
AATCATTTCAGCTGCAGGTAACCTCGATGTTAAGGAGGAAAGATCTGCCGCTTCTTATT
901 -----+-----+-----+-----+-----+-----+-----+ 960
TTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTCCTTCTAGACGGCGAAGAAATAA

b I I S A A G N F D V K E E R S A A S L L -
GCAGTTGTCGGTAATGCCAGTGATTTTATGGACGGAACCTAATAACTTGACAGC
961 -----+-----+-----+-----+-----+-----+-----+ 1020
CGTCAACAGGCCATTACGGTCACTAAAAAGTATACCTGCCCTGAGTTATTGAAACTGTCG

b Q L S G N A S D F S Y G R N S I T L T A -
ATCACGATAATATATTAATTAAATGATAGCAATCTTACTGGGCTGTGCCACATAAGATT
1021 -----+-----+-----+-----+-----+-----+-----+ 1080
TAGTCGTATTATATAATTAAATTTACTATCGTTAGAATGACCCGACACGGTGTATTCTAA

b S A * -
GCTATTTGGAGTCATAATGGATTCTTGTCAAAAATTGATTATGGGTATACGCC
1081 -----+-----+-----+-----+-----+-----+-----+ 1140
CGATAAAAAACCTCAGTATTACCTAACAGTATTAACTAACCAATATGCGGG

TGGAGATTTAGCCAATACCATAACGTCTGTAAACCCGGAAGAAATTAAACATAGAT
1141 -----+-----+-----+-----+-----+-----+-----+ 1200
ACCTCTAAAATCGGGTATGGTATTGCAGAGACAATTGGCCTCTTTAATTGTATCTA

TTGACACAGACGGGACTGGCTGGGATTAACGTCACTGGTCTGCTGCGAAATCTTAG
1201 -----+-----+-----+-----+-----+-----+-----+ 1260
AACTGTGTCTGCCCTGACCAGACCCCTATTGCACTAACGAACGACGACGCTTAGAAATC

AACTAAAGGTAAAACAGGTAAAAAAACAATTGACCGATTAAACTTATTCTGCCCCG
1261 -----+-----+-----+-----+-----+-----+-----+ 1320
TTGATTTCCATTTGTCCATTGGTAACTGGCTAACATTGAAATAAGAGACGGGC

CATTAGTCTGGAGAGAGGATGGACGTCATTATTCTGACTAAAGTCAGTAAAGAACAA
1321 -----+-----+-----+-----+-----+-----+-----+ 1380
GTAATCAGACCTCTCCTACCTGCAGTAAATAAGACTGATTTCAGTCATTCTCGTT

Fig. 7 (cont.)

Fig. 7 (cont.)

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Cutting enzymes:

BamHI	BglII	EagI	EcoRI	HindIII	NcoI	NheI	SalI
SfiI	XbaI	XmaI					

Not cutting enzymes:

None

Fig. 7 (cont.)

pVamyHYA map

With 8 enzymes: NCOI PSTI SALI HINDIII SFII BAMHI NOTI ECORI PSTI

ACTATAGGGAGACCACAAACGGTTCCCTCTAGAAATAATTTGTTAACCTTAAGAAGGA
 1 -----+-----+-----+-----+-----+-----+-----+ 60
 TGATATCCCTCTGGTGTGCCAAAGGGAGATCTTATTAAAACAATTGAAATTCTTCCT

 NcoI
 |
 GATATATCCATGGCTCAGGTGCAGCTGGTGGAGTCTGGGGAGGCTCGGTGCAGGCTGG
 61 -----+-----+-----+-----+-----+-----+-----+ 120
 CTATATAGGTACCGAGTCCACGTCGACCACCTCAGAACCCCTCCGAGCCACGTCCGACCC

 a M A Q V Q L V E S W G G S V Q A G -
 |=> VHH amylase

 GGGTCTCTGAGACTCTCCTGCACAGCCCCCTGGATTCACCTCCAATAGCTGCCGCATGGAC
 121 -----+-----+-----+-----+-----+-----+-----+ 180
 CCCAGAGACTCTGAGAGGACGTGTCGGGACCTAAGTGGAGGTTATCGACGGCGTACCTG

 a G S L R L S C T A P G F T S N S C R M D -

 PstI
 |
 TGGTACCGCCAGGCTGCAGGGAAAGCAGCGCAGTGGGTCTCATCTATTAGTACTGATGGT
 181 -----+-----+-----+-----+-----+-----+-----+ 240
 ACCAAGGCGGTCCGACGTCCCTCGTCGCGCTACCCAGAGTAGATAATCATGACTACCA

 a W Y R Q A A G K Q R E W V S S I S T D G -

 CGCACAAAGCTATGCAGACTCCGTGAAGGGCCGATTACCACATCTCAAAGACAAAGCCAAG
 241 -----+-----+-----+-----+-----+-----+-----+ 300
 GCGTGTTCGATACGTCTGAGGCACCTCCCGCTAAGTGGTAGAGGTTCTGTTCGGTTCT

 a R T S Y A D S V K G R F T I S K D K A K -

 GACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGCCATCTATTACTGT
 301 -----+-----+-----+-----+-----+-----+-----+ 360
 CTGTGCCACATAGACGTTACTTGTGGACTTGGACTCCTGTGCCGGTAGATAATGACA

 a D T V Y L Q M N S L K P E D T A I Y Y C -

 GCCGTGAGGACGAATGGGTATCGTCCGCAATCTCACGAATTCGCTACTGGGGCCGGGG
 361 -----+-----+-----+-----+-----+-----+-----+ 420
 CGGCACCTCGCTTACCCATAGCAGGCGTAGAGTGCTTAAAGCGATGACCCCGGGCCCC

 a A V R T N G Y R P Q S H E F R Y W G P G -

Fig. 8

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SfiI SalI
| |
421 ACCCAGGTACCGTCTCCTAACGGCCTCGGGGGCCGCGTCGACGCCCGGGGTGCGCCG
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480
TGGGTCCAGTGGCAGAGGAGTTGCCGGAGCCCCCGGCAGCTGCGGGCCCCACGCGGC

a T Q V T V S S T A S G A A S T P G G A P -
|=> E-tag
BamHI
|
481 GTGCCGTATCGGATCCGCTGGAACC GGCGGGgAAAATtCTCTGCTAAAAATGTATTAA
-----+-----+-----+-----+-----+-----+-----+-----+-----+ 540
CACGGCATAGGCCTAGGCGACCTTGCCGGCCcTTTTAaGAGAACGATTTTACATAAT

a V P Y P D P L E P A G E N S L A K N V L -
|=> C-hlyA

TCCGGTGGAAAAGGTAAATGACAAGTTGACGGCAGTGAGGGAGCAGACCTGCTGATGGC
541 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 600
AGGCCACCTTTCCATTACTGTTCAACATGCCGTCACTCCCTCGTCTGGACGAACCTACCG

a S G G K G N D K L Y G S E G A D L L D G -
GGAGAAGGGAAATGATCTTCTGAAAGGTGGATATGGTAATGATATTATCGTTATCTTCA
601 -----+-----+-----+-----+-----+-----+-----+-----+ 660
CCTCTCCCTTACTAGAAGACTTCCACCTATACCATTACTATAATAGCAATAGAAAGT

a G E G N D L L K G G Y G N D I Y R Y L S -
GGATATGGCCATCATATTATTGACGATGAAGGGGGAAAGACGATAAAACTCAGTTAGCT
661 -----+-----+-----+-----+-----+-----+-----+-----+ 720
CCTATACCGGTAGTATAACTGCTACTCCCCCTTCTGCTATTGAGTCAAATCGA

a G Y G H H I I D D E G G K D D D K L S L A -
GATATAGATTTCCGGGACGTGCTTTAACGAGAAGGGAAATGACCTCATTATGTATAAA
721 -----+-----+-----+-----+-----+-----+-----+ 780
CTATATCTAAAGGCCCTGCAACGGAAATTGCTTCCCTTACTGGAGTAATACATATT

a D I D F R D V A F K R E G N D L I M Y K -
GCTGAAGGTAAATGTTCTTCTATTGGCCACAAAAATGGTATTACATTTAAAAACTGGTTT
781 -----+-----+-----+-----+-----+-----+-----+ 840
CGACTTCCATTACAAGAAAGATAACCGGTGTTTACCATATGTAATTTGACCAAA

a A E G N V L S I G H K N G I T F K N W F -
GAAAAAGAGTCAGATGATCTCTCTAACATCAGATAGAGCAGATTTGATAAAAGACGGC
841 -----+-----+-----+-----+-----+-----+-----+ 900
CTTTTCTCAGTCTACTAGAGAGATTAGTAGTCTATCTCGTCTAAAACATTCTGCCG

a E K E S D D L S N H Q I E Q I F D K D G -
AGGGTAATCACACCAGATTCTCTTAAAAAGCATTTGAATATCAGCAGAGTAATAACAAG
901 -----+-----+-----+-----+-----+-----+-----+ 960
TCCCATTAGTGTGGTCTAAGAGAATTTCGTAACCTATAGTCGTCTCATTATTGTT

Fig. 8 (cont.)

a R V I T P D S L K K A F E Y Q Q S N N K -
 961 GTAAAGTTATGTGTATGGACATGATGCATCAACTTATGGGAGCCAGGACAATCTTAATCCA
 CATTCATAACACATAACCTGTAACGTAGTTGAATACCCCTCGGTCTGTTAGAATTAGGT 1020

a V S Y V Y G H D A S T Y G S Q D N L N P -
 1021 TTAATTAAATGAAATCAGCAAAATCATTTCAGCTGCAGGTAACCTCGATGTTAAGGAGGA
 AATTAATTACTTTAGTCGTTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTCTT 1080

a L I N E I S K I I S A A G N F D V K E E -
 1081 AGATCTGCCGCTCTTATTGCAGTTGTCGGTAATGCCAGTGATTTTCATATGGACGG
 TCTAGACGGCGAAGAAAATAACGTCAACAGGCCATTACGGTCACTAAAAAGTATACTGCC 1140

a R S A A S L L Q L S G N A S D F S Y G R -
 1141 AACTCAATAACTTGACAGCATCAGCATAATATATTAAATTAAATGATAGCAATCTTACT
 TTGAGTTATTGAAACTGTCGTAGTCGTATTATATAATTAAATTACTATCGTTAGAATGA 1200

a N S I T L T A S A * -
 1201 GGGCTGTGCCACATAAGATTGCTATTTGGAGTCATAATGGATTCTTGTCAATAAAT
 CCCGACACGGGTGTATTCTAACGATAAAAAACCTCAGTATTACCTAACGAGACAGTATTAA 1260

1261 TGATTATGGTTATACGCCCTGGAGATTAGCCAATACCATAACGTCTCTGTTAACCC
 ACTAATACCAATATGCGGGACCTCTAAATCGGGTATGGTATTGCAGAGACAATTGGG 1320

1321 GGAAGAAATTAAACATAGATTGACACAGACGGACTGGTCTGGGATTAACGTATGGTT
 CCTCTTTAATTGTATCTAAACTGTCGTGCCCTGACCAGACCTAACGCAGTACCAA 1380

1381 GCTTGCTGCGAAATCTTAGAACTAAAGGTAAAACAGGTAAAAAAACAATTGACCGATT
 CGAACGACGCTTAGAAATCTGATTTCCATTGTCCATTGGTTACTGGCTAA 1440

1441 AAACTTTATTCTCTGCCCGCATTAGTCTGGAGAGAGGATGGACGTCAATTATCTGAC
 TTTGAAATAAGAGACGGCGTAATCAGACCTCTCCTACCTGCAGTAAATAAGACTG 1500

Fig. 8 (cont.)

1501 TAAAGTCAGTAAAGAAGCAAACAGATATCTTATTCTGATCTGGAGCAGCGAAATCCCCG
 1560 ATTCAGTCATTCTCGTTGTCTAGAATAAAGACTAGACCTCGTCGCTTAGGGGC

1561 TGTTCTCGAACAGTCTGAGTTGAGGCCTTATATCAGGGCATATTATTCTATCGCTTC
 1620 ACAAGAGCTTGTCAGACTCAAACCCGAATATAGTCCCGTATAATAAGAATAGCGAAG

1621 CCGTTCTCTGTTGCCGGAAACTGGCGAAATTGACTTACCTGGTTATTCCGCCAT
 1680 GGCAAGAAGACAACGGCCCTTGACCGCTTAAACTGAAATGGACCAAATAAGGACGGTA

1681 TATAAAATACAGGAGAATATTATTGAAACCCCTGTTGTCTGTTTTTACAATTATT
 1740 ATATTATGTCCTCTTATAAAACTTGGAACACACAGACAAAAATGTTAATAA

1741 TGCATTAATAACCCCCCTTTTTTCAGGTGGTTATGGACAAAGTATTAGTCACAGGGG
 1800 ACGTAATTATTGGGGGGAAAAAAAGTCACCAATACCTGTTCATAAATCACGTGTCCCC

1801 ATTTCAACTCTTAATGTTATTACTGTCGATTATCTGTTGTGGTGTGTTGAGATTAT
 1860 TAAAAGTTGAGAATTACAATAATGACAGCGTAATAGACAACACCACAAACTCTAATA

1861 ACTCAGCGTTAAGAACTTACATTTGCACATAGTACAAGTCGGATTGATGTTGAGTT
 1920 TGAGTCGCAAATTCTGAATGTTAAACGTGTATCATGTTAGCCTAACTACAACCAA

1921 GGGGCCAAACTCTCCGGCATTTACTGGCGCTACCGATCTTATTTGAGAGTCGTCG
 1980 CCCACGGTTGAGAAGGCCGTAAATGACCGCGATGGCTAGAGAATAAAACTCTCAGCAGC

1981 TGTTGGTGTACTGTTGCCAGGGTAAGAGAATTAGACCAAGATCCGTAATTTCTGACAGG
 2040 ACAACCACTATGACAACGGTCCCATTCTTAATCTGGTAGGCATTAAAAGACTGTCC

2041 ACAGGCATTAACATCTGTTCTGGACTTATTATTTCATTCAATTGCGGTAAATGTG
 2100 TGTCCGTAATTGAGACAAGACCTGAATAATAAAAGTAAGTATAAAAACGCCATTACAC

Fig. 8 (cont.)

HindIII

GTATTACAGTCAAAGCTTACTCTGGTGATCTTATTTCGCTGCCTGTTATGCTGCATG
 2101 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2160
 CATAATGTCAGGTTCGAATGAGACCACTAGAATAAAAGCGACGGAACAATACGACGTAC

GTCTGTTTATTAGCCCCATTTGCGACGTCGCCTGATGATAAGTTCACCGGAATGC
 2161 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2220
 CAGACAAAATAATCGGGTAAACGCTGCAGCGGAACACTATTCAAAAGTGCCTTACG

GGATAATCAATCTTCCTGGTGGAACATCAGTCACGGGATTAACACTATAAAAGCTATGGC
 2221 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2280
 CCTATTAGTTAGAAAGGACCACCTAGTCAGTGCCGTAATTGTGATATTCGATACCG

PstI

AGTCTCACCTCAGATGACGAACATATGGGACAAACAATTGGCAGGATATGTTGCTGCAGG
 2281 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2340
 TCAGAGTGGAGTCACTGCTGTATACCTGTTGTTAACCGTCTATACAACCGACGTCC

CTTCAAAGTACAGTATTAGCAACCATTGGCAACAAGGAATACAGTTAATACAAAAGAC
 2341 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2400
 GAAGTTTCACTGTCATAATCGTTGGTAACCAAGTTGTTCTTATGTCATTATGTTTCTG

TGTTATGATCATCAACCTGTGGTGGAGCACACCTGGTTATTCGGGGATTTAAGTAT
 2401 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2460
 ACAAACTAGTAGTTGGACACCAACCCCTCGTGTGGACCAATAAGGCCCTAAATTCTATA

TGGTCAGTTAATTGCTTTAATATGCTGCTGGCAGATTGTTGACCCGTTATCGCCT
 2461 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2520
 ACCAGTCATTAACGAAAATTATACGAACGACCAGTCTAACACGTGGCCAATAAGCGGA

TGCACAAATCTGGCAGGATTCAGCAGGTTGGTATATCAGTTACCGCCTGGTGATGT
 2521 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2580
 ACGTGTTAGACCGTCCTAAAGTCGTCAACCATAAGTCATGGCCGGAACCACTACA

GCTTAACTCTCAAAC TGAAAGTTATCATGGGAAACTGGCATTACGGAAATTATGGTGA
 2581 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2640
 CGAATTGAGAGGTTGACTTTCAATAGTACCCCTTGACCGTAATGGCCTTAATTACCACT

TATCACTTTCGTAATATCCGGTTTCGCTATAAGCCTGACTCTCCGGTTATTTAGATAA
 2641 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2700
 ATAGTGAAGCATTATAGGCCAAGCGATATTGGACTGAGAGGCCAATAAAATCTATT

TATCAATCTCAGTATTAAGCAGGGGGAGGTATTGGTATTGTCGGACGTTCTGGTCAGG
 2701 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 2760
 ATAGTTAGAGTCATAATTGTCCCCCCTCCAATAACCATAACAGCCTGCAAGACCAAGTCC

Fig. 8 (cont.)

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AAAAAGCACATTAACATAAAATTAAATTCAACGTT
2761 -----+-----+---+--- 2792
TTTTCGTGTAAATTGATTAAAGTTGCAA

Cutting enzymes:

BamHI HindIII NcoI PstI SalI SfiI

Not cutting enzymes:

EcoRI NotI

Fig. 8 (cont.)

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PVamyZHLYA map

With 11 enzymes: ECORI SPHI PSTI NCOI NHEI NDEI BAMHI HINDIII
SALI SFII NOTI

ACTATAGGGAGACCACAAACGGTTCCCTCTAGAAATAATTTGTTAACCTTAAGAAGGA
1 -----+-----+-----+-----+-----+-----+ 60
TGATATCCCTCTGGTGTGCCAAAGGGAGATCTTATTAAAACAATTGAAATTCTTCCT

NcoI
|
GATATATCCATGGCTCAGGTGCAGCTGGAGTCTTGGGGAGGCTCGGTGCAGGCTGG
61 -----+-----+-----+-----+-----+-----+ 120
CTATATAGGTACCGAGTCCACGTCGACCACCTCAGAACCCCTCCGAGCCACGTCCGACCC

a M A Q V Q L V E S W G G S V Q A G -
|=> VHH amylase

GGGTCTCTGAGACTCTCCTGCACAGCCCCCTGGATTCACCTCCAATAGCTGCCGCATGGAC
121 -----+-----+-----+-----+-----+-----+ 180
CCCAGAGACTCTGAGAGGACGTGCGGGACCTAAGTGGAGGTTATCGACGGCTACCTG

a G S L R L S C T A P G F T S N S C R M D -

PstI
|
TGGTACCGCCAGGCTGCAGGGAAGCAGCGCAGTGGTCTCATCTATTAGTACTGATGGT
181 -----+-----+-----+-----+-----+-----+ 240
ACCAAGGCGGTCCGACGTCCCTCGTCGCGCTCACCCAGAGTAGATAATCATGACTACCA

a W Y R Q A A G K Q R E W V S S I S T D G -

CGCACAAAGCTATGCAGACTCCGTGAAGGGCGATTACCATCTCAAAGACAAAGCCAAG
241 -----+-----+-----+-----+-----+-----+ 300
GCGTGTTCGATACGTCTGAGGCACCTCCCGCTAAGTGGTAGAGGTTCTGTTCGGTTC

a R T S Y A D S V K G R F T I S K D K A K -

GACACGGTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGCCATCTATTACTGT
301 -----+-----+-----+-----+-----+-----+ 360
CTGTGCCACATAGACGTTACTTGTGGACTTGGACTCTGTGCCGGTAGATAATGACA

a D T V Y L Q M N S L K P E D T A I Y Y C -

GCCGTGAGGACGAATGGGTATCGTCCGCAATCTCACGAATTCGCTACTGGGGCCCGGG
361 -----+-----+-----+-----+-----+-----+ 420
CGGCACCTCGCTTACCCATAGCAGGCGTTAGAGTGCTAAAGCGATGACCCGGGCCCC

Fig. 9

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a A V R T N G Y R P Q S H E F R Y W G P G -

SfiI SalI

421 | |
ACCCAGGTACCGTCTCCTCAACGGCTCGGGGCCGCGTCGACGTCCGGCGTCCGAAG
-----+-----+-----+-----+-----+-----+-----+-----+ 480
TGGTCCAGTGGCAGAGGAGTTGCCGGAGCCCCGGCGCAGCTGCAGGCCAGGCTTC

a T Q V T V S S T A S G A A S T S G G P K -
|=> Ig hinge

481 |
CCTTCCACTCCGCCGGTCTCCGTATGAAACAGCTGGAAGACAAAGTAGAAGAGCTC
-----+-----+-----+-----+-----+-----+-----+ 540
GGAAGGTGAGGCCAGAACGGCATACTTGTGACCTCTGTTCATCTCGAG

a P S T P P G S S R M K Q L E D K V E E L -
|=> Leucine Zipper GCN4

HindIII

541 |
CTTAGCAAGAACTACCATCTAGAAAACGAGGTAGCTCGTCTGAAAAAGCTTGTGGTGAA
-----+-----+-----+-----+-----+-----+-----+ 600
GAATCGTTCTTGATGGTAGATCTTGCTCCATCGAGCAGACTTTCGAACAAACCACTT

a L S K N Y H L E N E V A R L K K L V G E -

SalI

601 |
CGTGGTGGTACCATCACCATCACCATGCGTCGACGCCGGGGTGCGCCGGTGCCTAT
-----+-----+-----+-----+-----+-----+-----+ 660
GCACCAACCAGTGGTAGTGGTAGTGGTAGCGCAGCTGCCGGCCCCACGCCAACGGCATA

a R G G H H H H H H A S T P G G A P V P Y -
|=> 6xhis tag |=> E-tag

BamHI

661 |
CCGGATCCGCTGGAACCGGCCGGgAAAAttCTCTTGCTAAAATGTATTATCCGGTGG
-----+-----+-----+-----+-----+-----+-----+ 720
GGCTAGGCGACCTTGCCGGCCcTTTTAaGAGAACGATTTACATAATAGGCCACCT

a P D P L E P A G E N S L A K N V L S G G -
|=> C-hlyA

721 |
AAAGGTAAATGACAAGTTGTACGGCACTGAGGGAGCAGACCTGCTTGTGGCGGAGAAGGG
-----+-----+-----+-----+-----+-----+-----+ 780
TTTCCATTACTGTTCAACATGCCGTACTCCCTCGTCTGGACGAACCTACCGCCTCTCCC

a K G N D K L Y G S E G A D L L D G G E G -

Fig. 9 (cont.)

AATGATCTTCTGAAAGGTGGATATGGTAATGATATTATCGTTATCTTCAGGATATGGC
 781 -----+-----+-----+-----+-----+-----+-----+ 840
 TTACTAGAAGACTTCCACCTATACCATTACTATAAATAGCAATAGAAAGTCCTATACCG

a N D L L K G G Y G N D I Y R Y L S G Y G -

CATCATATTATTGACGATGAAGGGGGAAAGACGATAAAACTCAGTTAGCTGATATAGAT
 841 -----+-----+-----+-----+-----+-----+-----+ 900
 GTAGTATAATAACTGCTACTTCCCCCTTCTGCTATTTGAGTCAGACTATATCTA

a H H I I D D E G G K D D K L S L A D I D -

TTCCGGGACGTTGCCTTTAACCGAGAAGGAAATGACCTCATTATGTATAAAGCTGAAGGT
 901 -----+-----+-----+-----+-----+-----+-----+ 960
 AAGGCCCTGCAACGAAATTGCTCTCCCTACTGGAGTAATACATATTCGACTTCCA

a F R D V A F K R E G N D L I M Y K A E G -

AATGTTCTTCTATTGCCACAAAATGGTATTACATTAAAAACTGGTTGAAAAAGAG
 961 -----+-----+-----+-----+-----+-----+-----+ 1020
 TTACAAGAAAGATAACCGGTGTTTACCATATGAAATTTTGACCAAACCTTTCTC

a N V L S I G H K N G I T F K N W F E K E -

TCAGATGATCTCTAATCATCAGATAGAGCAGATTTTGATAAAAGACGGCAGGGTAATC
 1021 -----+-----+-----+-----+-----+-----+-----+ 1080
 AGTCACTAGAGAGATTAGTAGTCTATCTCGTCTAAAACATTTCTGCCGTCCCATTAG

a S D D L S N H Q I E Q I F D K D G R V I -

ACACCGAGATTCTTAAAAAAGCATTTGAATATCAGCAGAGTAATAACAAGTAAGTTAT
 1081 -----+-----+-----+-----+-----+-----+-----+ 1140
 TGTGGTCTAAGAGAATTTTCGTAACCTATAGTCGCTCATTATTGTTCCATTCAATA

a T P D S L K K A F E Y Q Q S N N K V S Y -

GTTATGGACATGATGCATCAACTTATGGGAGCCAGGACAATCTTAATCCATTAATTAAT
 1141 -----+-----+-----+-----+-----+-----+-----+ 1200
 CACATACCTGTACTACGTAGTTGAATACCCCTCGGTCTGTTAGAATTAGGTAATTAATTA

a V Y G H D A S T Y G S Q D N L N P L I N -

Fig. 9 (cont.)

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PstI

1201 GAAATCAGCAAAATCATTCAGCTGCAGGTAACCTCGATGTTAAGGAGGAAGATCTGCC
CTTAGTCGTTTAGTAAAGTCGACGTCCATTGAAGCTACAATTCCCTTTCTAGACGG 1260

a E I S K I I S A A G N F D V K E E R S A -

NdeI

1261 GCTCTTTATTGCAGTTGTCCGTAATGCCAGTGATTTCATATGGACGGAACCTCAATA
CGAAGAAAATAACGTCAACAGGCCATTACGGTCACTAAAAAGTATACCTGCCTTGAGTTAT 1320

a A S L L Q L S G N A S D F S Y G R N S I -

ACTTTGACAGCATCAGCATAATATATTAATTAAATGATAGCAATCTTACTGGGCTGTGC
1321 TGAAACTGTCGTAGTCGTATTATATAATTAAATTACTATCGTTAGAATGACCCGACACG 1380

a T L T A S A * -

CACATAAGATTGCTATTTGGAGTCATAATGGATTCTGTCATAAAATTGATTATGG
1381 GTGTATTCTAACGATAAAAAAACCTCAGTATTACCTAACAGTATTTAACTAACCTAC 1440

GTTATACGCCCTGGAGATTTAGCCAATACCATAACGTCTCTGTTAACCGGAAGAAAT
1441 CAATATGCGGGACCTCTAAATCGGGTTATGGTATTGCAGAGACAATTGGCCTCTTA 1500

TAAACATAGATTGACACAGACGGACTGGCTGGGATTAACGTACGGTGCTGCTGC
1501 ATTTGTATCTAACTGTGCTGCCCTGACCAGACCTAACCGTTAACGACG 1560

GAAATCTTGAACTAAAGGAAACAGGTAAAAACAGGTAAAAAAACAATTGACCGATTAAACTTAT
1561 CTTTAGAAATCTGATTTCCATTGGTCACTGGCTAACGGCTAACGGAAATA 1620

TTCTCTGCCGCATTAGTCTGGAGAGAGGATGGACGTCAATTCTGACTAAAGTCAG
1621 AAGAGACGGGCGTAATCAGACCTCTCCTACCTGCAGTAAATAAGACTGATTTCAGTC 1680

TAAAGAAGCAAACAGATATCTTATTCGATCTGGAGCAGCGAAATCCCCGTGTTCTCGA
1681 ATTTCTCGTTGTCTATAGAATAAGACTAGACCTCGTCGCTTAGGGGCACAAGAGCT 1740

Fig. 9 (cont.)

1741 ACAGTCTGAGTTGAGGCCGTATATCAGGGCATATTATTCTTATCGCTTCCGTTCTTC
 1800 TGTCAGACTCAAACCTCCGAATATACTGCCCCGTATAATAAGAATAGCGAAGGGCAAGAAG

 1801 TGTTGCCGGAAACTGGCGAAATTGACTTACCTGGTTTATTCTGCCATTATAAAAATA
 1860 ACAACGGCCCTTGACCCTTAAACTGAAATGGACCAAATAAGGACGGTAATATTTAT

 1861 CAGGAGAATATTATTGAAACCCCTGTTGTCTGTTTTTACAATTATTGCATTAAT
 1920 GTCCTCTTATAAATAACTTGGGAACAACACAGACAAAAAAATGTTAATAAACGTAATTA

 1921 AACCCCCCTTTTTCAGGTGGTTATGGACAAAGTATTAGTCACAGGGGATTTCAAC
 1980 TTGGGGGGAAAAAAAGTCCACCAATACCTGTTCATATACTCACGTGCCCCCTAAAGTTG

 1981 TCTTAATGTTTACTGTCGATTATCTGTTGTTGAGATTATACTCAGCGG
 2040 AGAATTACAATAATGACAGCGTAATAGACAACACCACAACTCTAATATGAGTCGCC

 2041 TTTAAGAACCTACATTTGACACATAGTACAAGTCGGATTGATGTTGAGTTGGTGCAA
 2100 AAATTCTTGAATGAAAAACGTGTATCATGTTCAGCCTAACTACAACCAACCGTT

 2101 ACTCTTCCGGCATTACTGGCGCTACCGATCTCTTATTTGAGAGTCGTCGTGTTGGA
 2160 TGAGAAGGCGTAAATGACCGCGATGGCTAGAGAATAAAACTCTCAGCAGCACACCACT

 2161 TACTGTTGCCAGGGTAAGAGAATTAGACCAAGATCCGTATTTCTGACAGGACAGGCATT
 2220 ATGACAACGGTCCCATTCTTAATCTGGCTAGGCATTAAGACTGTCCTGTCGTAA

 2221 AACATCTGTTCTGGACTTATTATTTCATTCATTTTGCCTAATGTTGAGAGTCGTCGTGTT
 2280 TTGTAGACAAGACCTGAATAATAAAAGTAAGTATAAAACGCCATTACACCATAATGTC

 HindIII
 |
 2281 TCCAAAGCTTACTCTGGTGTCTTATTTCGCTGCCTTGTATGCTGCATGGCTGTTT
 2340 AGGTTTCAATGAGACCACTAGAATAAAAGCGACGGAACAATACGACGTACCGACAAAA

 2341 TATTAGCCCCATTGCGACGTCGCCTTGTGATGATAAGTTTACGGAAATGCGGATAATCA
 2400 ATAATCGGGTAAACGCTGCAGCGGAACACTACTATTCAAAAGTGCCTTACGCCTATTAGT

Fig. 9 (cont.)

2401 ATCTTCTGGTGAATCAGTCACGGGATTAACACTATAAAAGCTATGGCAGTCTCAC
 2460 TAGAAAGGACCACCTTAGTCAGTGCGCTAATTGTGATATTTCGATACCGTCAGAGTGG

NdeI	PstI
TCAGATGACGAACATATGGGACAAACAATTGGCAGGATATGTTGCTGCAGGCTCAAAGT	

2461 AGTCTACTGCTTGTATAACCCCTGTTAACCGTCCTATACAACGACGTCCGAAGTTCA

2521 GACAGTATTAGCAACCATTGGTCAACAAGGAATACAGTTAACAAAAGACTGTTATGAT
 2580 CTGTCATAATCGTGGTAACCAGTTGTTCTTATGTCAATTATGTTCTGACAATACTA

2581 CATCAACCTGTGGTGGGAGCACACCTGGTTATTCGGGGATTTAAGTATTGGTCAGTT
 2640 GTAGTTGGACACCAACCCTCGTGTGGACCAATAAGGCCCTAAATTCAACCAGTCAA

2641 AATTGCTTTAATATGCTGGTCAGATTGTTGCACCGGTTATCGCCTTGACAAAT
 2700 TTAACGAAAATTATACGAACGACCAGTCAACACGTGGCAATAAGCGAACGTGTTA

2701 CTGGCAGGATTCACCAGGGTGGTATATCAGTTACCCGCTTGGTGATGTGCTTAAC
 2760 GACCGTCTAAAGGTCGTCCAACCATAAGTCAATGGCGGAACCACTACACGAATTGAG

2761 TCCAACGTAAAGTTATCATGGAAACTGGCATTACCGAAATTAAATGGTGATATCACTT
 2820 AGGTTGACTTTCAATAGTACCCCTTGACCGTAATGGCCTTAATTACCACTATAAGTGA

2821 TCGTAATATCCGGTTTCGCTATAAGCCTGACTCTCCGGTTATTTAGATAATATCAATCT
 2880 AGCATTATAGGCCAAAGCGATATTCGGACTGAGAGGCCAATAAAATCTATTATAGTTAGA

2881 CAGTATTAAGCAGGGGGAGGTTATTGGTATTGTCGGACGTTCTGGTCAGGAAAAAGCAC
 2940 GTCATAATTGCCCCCTCCAATAACCATAACAGCCTGCAAGACCAAGTCCTTTCTG

2941 ATTAACATAATTCAACGTT
 2963 TAATTGATTAAATTAAAGTTGCAA

Fig. 9 (cont.)

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Cutting enzymes:

BamHI HindIII NcoI NdeI PstI SalI SfiI

Not cutting enzymes:

EcoRI NheI NotI SphI

Fig. 9 (cont.)

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